



IBP/UCLA CLOACAL SWAB AND FEATHER SAMPLING PROTOCOL

April 7, 2009

The Institute for Bird Populations (IBP) is collaborating with the Center for Tropical Research at UCLA to collect samples of avian influenza virus (AIV) to identify transmission paths in North American migratory landbirds, and to further the goal of developing custom vaccines against Influenza A. Our objective is to facilitate the collection of viral samples, through cloacal swabbing, from birds captured at MAPS (Monitoring Avian Productivity and Survival) stations during the summers of 2006-2009 and at MoSI (Monitoreo de Supervivencia Invernal) and MAWS (Monitoring Avian winter Survival) stations during the winters of 2006-2009. IBP is also working with the Landbird Migration Monitoring Network of the Americas (LaMMNA) to incorporate cloacal-swab samples from landbird migration monitoring efforts.

During the 2009 MAPS season we will continue sampling from all species except hummingbirds. We are hoping that station operators will sample as many of these birds as possible throughout the MAPS season. In order to gain information on connectivity between breeding and wintering grounds (and thus potential pathways for the spread of avian influenza) we ask that feather samples be collected for DNA and stable isotope analyses from all individuals for which cloacal swab samples are collected. This document provides information on UCLA/IBP's sampling strategy and details on cloacal-swabbing and feather-pulling techniques to be employed during the 2009 MAPS season.

Sampling Kits

All participants in this effort will be provided with cloacal swab sampling kits, which will be used to take cloacal swab samples from captured birds. Cloacal swab kits will be mailed to each operator by UCLA. Each kit will contain:

- Safety equipment (i.e., antiseptic skin cleanser, gloves, safety glasses, and respirator masks)
- Individually packaged 1-mm Dacron swabs
- Plastic vials containing 1ml of ethanol (SAFETY NOTE: ETHANOL IS FLAMMABLE)
- Barcoded labels to place on sample vials
- A pair of diagonal cutting pliers
- A fine-point, felt-tipped pen
- A 35-mm film canister (for holding the lid of the plastic vial while the swab is being placed into the vial)
- Labeled envelopes for feather samples
- Cloacal swab sampling data sheets for recording cloacal swab data
- Extra plastic bags for packing vials for return shipment
- A return prepaid shipping form for sample vials and feather envelopes and datasheets

Registration documents, sampling protocol, safety guidelines, and data forms can be found on the IBP website (<http://www.birdpop.org/aimaps.htm>). If you have already have a registration form on file for your station there is no need to fill one out again.

Cloacal swab sampling kits will be mailed by UCLA to all participating station operators during April and May 2009. Each sampling kit will contain 100 vials and 105 1mm swabs (five extra swabs in case of contamination; see below). If you already have equipment from previous years of sampling and do not need more equipment, let us know. **Do not however, use any guanadine filled vials from the 2006-2007 seasons.**

Your Safety and Protection

To date, no human has been diagnosed as having become infected with avian flu from contact with a live wild bird, and the highly pathogenic H5N1 variant has not been detected in the Western Hemisphere. It is important to realize, however, that a great many of the birds you handle and band (and have handled and banded in past years) have carried other forms of avian influenza. Thus, normal hygiene precautions, that include sanitizing hands before hand to mouth contact and before touching food, should always be taken when banding birds.

In order to protect MAPS operators, we require that all persons handling birds apply antiseptic skin cleanser to their hands immediately after all birds captured on each net run are released. To further minimize the probability of bird-to-bird transfer of any avian disease, we suggest that operators apply antiseptic skin cleanser to their hands after each individual bird is handled, both when removing birds from the nets and when processing birds at the central banding location. Operators should rub a small amount of the skin cleanser thoroughly over their hands and then let their hands dry (which only takes a few seconds). Please remember to keep the spout of the hand cleanser closed when not in use to avoid evaporation. Operators should not rub their eyes, eat, drink, or smoke during the banding session and all food items should be kept well away from the area where birds are banded. Water should be consumed only after hands have been cleaned using the antiseptic skin cleanser or washed with soap and water. Safety gloves and safety glasses are provided in each kit for operators who would feel more comfortable using them. The respirator mask will only need to be used in areas where HPAI-H5N1 has been detected (as of this writing, nowhere in the Western Hemisphere).

Which Birds to Sample

As mentioned above, our goal is to sample as many individuals as possible. We are **especially** interested in European Starlings, Brown-headed Cowbirds, House Sparrows and House Finches. We request that you **not** sample hummingbirds (they are too small) or any unbanded bird. Because there is a limited time period during which active cloacal shedding of viruses occurs, recaptured birds, including those between periods within 2009, can be resampled to increase the probability of detecting any viruses or permit detection of new flu variants in the same individual. There is no need to sample from the same individual within the same period. During those times when large numbers of birds must be processed from a single net run, we recommend that viral and feather sampling be temporarily suspended in order to reduce handling and holding times for those birds.

Recommended Sampling Protocol

To avoid cross-contamination of samples, it is imperative to keep holding bags as clean as possible. Ideally, each holding bag should be used only once (to hold a single bird for cloacal swab sampling) during an entire day of MAPS or MoSI banding, and should be washed (using one cup of Chlorox per wash load) and dried between days of banding. We realize that some stations can capture large numbers of birds in a single day or pulse and may not have enough

holding bags to meet the above ideal situation. If a holding bag must be reused before being washed and dried, it must be thoroughly shaken out, turned inside-out, and shaken again before being used for another bird. If you must resort to shaking out a holding bag, please wear safety glasses and a respirator mask while shaking out the bag, and do it well away from the banding area and from other persons.

We recommend that all of the normal banding, processing, and data collection, including weighing the bird and collecting tail feathers, be completed before taking the cloacal swab sample. This will allow the bird to be released as soon as the swab sample has been collected.

The entire banding, processing, data collection, and cloacal swabbing procedure can be performed by a single person, although it can be helpful to have a second person present to record data. If two bird handlers are present, it may be useful for one person to complete the normal banding, processing, and data collection and hand the bird to the second handler to obtain the cloacal swab sample. Alternatively, each bird handler may completely process each bird from banding through cloacal swab sampling.

Feather Collection Protocol

When tail feathers are pulled, a small amount of skin cells remain attached to the quill of the feather. These skin cells are a valuable source of DNA that can be used to determine the population origin of an individual bird. Moreover, a portion of the feather itself can be used for stable isotope analyses, which can provide important information on the location (at least latitude) where the feather was grown. Researches at UCLA and elsewhere use the results of DNA and stable isotope analyses to investigate patterns of migratory connectivity in birds, that is, to determine wintering locations for populations of breeding birds and vice-versa.

We recommend that two tail feathers be collected during the banding process from each bird (excluding woodpeckers for which tail feathers are critical to their foraging ability) that is to be swabbed, prior to collecting the cloacal swab sample. There is no need to collect feathers more than once during the same season, even if the individual is sampled for AIV more than once during within-season recaptures (see above). Also, although we hope to get both AIV and feather samples from all individuals, the collection of one of these samples without the other will still provide valuable data.

Pluck the outer tail feather from one side of the bird's tail, and the central tail feather from the other side of the tail. To pluck the feathers, just hold them firmly, relatively close to the base, and pull gently. Do not touch the quill, as the DNA is extracted from the skin cells attached to it. Place the feathers from each bird into one of the pre-printed envelopes provided by UCLA. Fill out the requested information on the envelope: species name (4-letter code is fine), band number, date (please use letters for the month instead of numbers), location code, station code, feather pull, and, if possible, age, sex, and breeding condition (as determined by brood patch or cloacal protuberance). Please make a note if you notice that the central rectrix is of a different generation than the outer rectrix (e.g., alternate vs. basic or juvenal vs. formative feather).

Detailed Cloacal Swab Protocol

Virus cells are located in the gastrointestinal tract in birds and can be present on the cloaca walls and in feces. Correct cloacal swabbing provides a sample of cells from the inner surface of the cloacal wall, and is accomplished by inserting the swab into the cloaca and slowly rotating the swab to collect these cells.

To obtain a cloacal swab sample, take the following steps:

- 1) Hold the bird in the bander's grip and remove the Dacron swab from its package, being very careful to let nothing touch the tip of the swab
- 2) Use your fingers to secure the bird's legs and tail.
- 3) Blow on the vent of the bird to part the feathers, exposing the cloaca. An optical binocular magnifier can make it easier to see what you are doing.
- 4) Gently and slowly insert the Dacron head of the swab into bird's cloacal cavity. Remember that cloacal cavities of small birds can be very shallow; thus the swab head should not be inserted very far into the cloaca. Once the swab is initially inserted change its angle slightly such that the swab is more aligned with the bird's body and the head of the swab is pointing more toward the head of the bird. This will help prevent damage to the softer membranes along the back wall of the cloaca.
- 5) Gently twirl or rotate the swab back and forth 2-5 times to exfoliate (collect) cells from the cloacal wall.
- 6) Remove the swab from the cloacal cavity and release the bird.

To place the cloacal swab sample in a plastic vial and store the used vials, take the following steps:

- 1) While still holding the cloacal swab in your hand, remove the cap from a plastic vial and place the cap in the clean, empty film canister provided for this purpose. Continue to assure that nothing touches the tip of the cloacal swab.
- 2) Place the swab head-first into the ethanol in the plastic vial and briefly but rapidly spin the swab 2-5 times in the vial to suspend any collected material in the ethanol.
- 3) Pull the swab partially out of the ethanol and use the diagonal cutting pliers to cut the aluminum shaft of the swab near (above) the top of the Dacron swab head. This will allow the swab head to remain in the vial while you screw the cap back onto the vial and seal the contents. Tighten the cap as much as possible to prevent evaporation of the ethanol during storage.
- 4) Use the fine-point felt-tipped pen to mark the cap of the vial to indicate that it has been used, and to record the four-letter species code and band number on the vial.

- 5) Fill out the cloacal swab sampling data sheet by entering the band number, sample vial number (from the bar code), four-letter species code, age, sex, date, station, feather pull, and any relevant comments onto the data sheet. Don't forget to fill out the location code on top of the data sheet.
- 6) Place the used vial into a plastic bag with the other used vials and store them in a shaded, cool location until the end of the day's banding session.
- 7) At the end of the day of banding, store the plastic bag containing all used vials in a cool and dry location until all fifty vials in a given kit have been used (they do NOT need to be refrigerated).

Returning Completed Samples to UCLA

Finally, as soon as the season is over, seal the sample boxes of vials with two ziplock bags and ship the boxes back to UCLA, along with the associated feather samples and the completed cloacal swab sampling data sheets associated with those vials. Use the prepaid shipping form provided by UCLA.

Thank you very much for your participation in this important work!

Cloacal Swabbing – an illustrated protocol



Here a chickadee is held in preparation for cloacal swabbing (left). Note that one leg is held between the thumb and forefinger and the other leg is held between the forefinger and middle finger, while the little finger of the swabbing hand holds the base of the tail against the palm of the holding hand. This allows you to pose the swab (in this case a 1-mm swab) in preparation for locating the cloaca. Note the angle of the swab relative to the bird (see text).

The cloaca may be clearly visible as seen in this female chickadee (right). However, the cloaca may not be clearly visible until you blow gently on the vent area to move the feathers of the abdomen out of the way (below).



Once the cloaca is visible, as shown below, insert the swab into the opening, angle it back slightly (see above), and gently and rotate the swab back and forth 2-5 times to gather any material from the walls of the cloaca. Continue to blow gently on the abdomen so that the cloaca remains clearly visible throughout the procedure.



After you have removed the swab, place it in the vial containing ethanol and swirl it around 2-5 times. Cut off the excess wire and tighten the cap down tightly on the vial. Write the species code and band number on the vial. Record the bar code number, along with the band number, species code, age, sex, date, station, and any comments on the cloacal swab sample data sheet.